RESEARCHARTICLE



Comparative Study of He – Ne and Green Lasers Effect on Normal Human Blood Invitro Using FTIR Techniques

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ABSTRACT

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The contact of lasers with biomaterials like tissues, blood, and skin is a central field of research. Invitro irradiation of human blood with laser beams has been studied for decades, to understand the biostimulatory effects on the different blood cells. Laser tissue associations are determined by employing spectroscopic and cutting-edge microscopic technicality. In this study, blood was extracted under standard laboratory settings from healthy human subjects. The samples were exposed to He-Ne laser (wavelength λ = 650nm, power p= 100mW) and green laser (wavelength λ = 530nm, power p= 500mW).Complete blood count (CBC) and FTIR spectra for non-irradiated samples were compared to CBC and FTIR spectra of irradiated samples.During exposure to green laser light MCH and PCT were not affected while under He-Ne laser exposure all CBC parameters were affected. FTIR spectrum of normal blood laser irradiation indicated that O-H, C=O, C-O, NH, N=O and C-H.As the duration of exposure to He-Ne laser irradiation on whole blood is decreased for 10, 20, and 30 minutes, the absorption for all groups, except NH and CO absent peaks for 10 and 20 minutes, yet it is present in 30 minutes.When blood is exposed to green laser, all of the functional groups OH,C=O,CH and N=O are present, however for control 2 only peaks of CO after irradiation for 30 minutes are absent. The findings prove that green laser light therapy has a lesser effect on the blood component than He-Ne lasers.

Keywords: Helium Neon (He-Ne) laser, Green laser, FTIR, Healthy human blood samples, Negative control

1. INTRODUCTION

Laser beam irradiation has proven to be tremendously effective for biomedical and biological uses as well as in medical treatments(Di Giacomo et al., 2013, Mohammed and Hassan, 2021, Faber et al., 2005). Photodynamic and biostimulation treatment are used on photochemical interactions; which refers to the interface leading to laser consumption of cellular constituent such as endogenous chromophores and mitochondrial cytochromes. In 2005, Weber utilized green laser beams for intravascular blood treatment, which had not been done until then. The purpose was to excess blood energy integration through the assimilation of green laser beams, which can in turn effectively compliment red light. That is when the influence of green laser on the rheological prosperities of blood were revealed. As it became clear, these were more beneficial than red light(Eman, 2017).

The effects of laser light on the tissues then became an integral part of medical studies. This also led to fluorescence, chemical reactions, and thermal effects with thermal being the most versatile (Ansari et al., 2013, Choi and Welch, 2001, Barton et al., 2001, Lapotko, 2006). Consequently, this will lead to major transformations in the visual limitations of tissues and human blood, including diffusion, reflection, absorption efficiency, as well as the interactive index (n) (Jacques, 2013, Faber et al., 2005). Fourier Transform Infra Red (FTIR) and UVspectra vis spectroscopic approaches were taken in researching the

spectral differences in the serum of normal blood samples (Ghadage et al., 2015). In that study, blood was exposed to He-Ne laser (wave length Λ =632.8 nm, power =3mW). The FTIR spectra for the irradiated blood samples displayed substantial alterations (Ghadage Vijay and Lokare, 2018). Human blood under low-intensity He-Ne radiation leads to noticeable alterations in the IR and evident absorption spectra of the blood and erythrocytes. These variations are the result of the partial photo detachment of the hemoglobin ligand (Weber et al., 2007).

Physical transformations of nucleic acids, lipids and proteins, are documented as a result of FTIR. The FTIR spectrum is used to identify the composite structures of globular proteins and biomolecules in blood(Itoh et al., 2000). Changes that can occur in a cell, such as the inactivation of enzymes, metabolic rate variations, and derangements of the clotting system may modify erythrocyte properties at a molecular and cellular level. Laser therapy, it has been discovered, decreases the thickness of blood and, as a result, augments the electrophoretic movement of erythrocytes. Laser beam also boosts modifications in the RBC membrane, which are connected to changes in the physical conditions of both erythrocyte membrane proteins and the bilayer of lipid, subsequently leading to changes in the motion of ion pumps (Siposan and Lukacs, 2000). Irradiation settings, energy effluence, wave length and length of timedefine the photo thermal consequences of laser light on blood cells. The defensive effects of the low power He-Ne laser on the damage of red blood cells is documented (Ghadage and Zaware, 2018, Zahra Al Timimi and Jafri, 2011). The main purpose of this study is to determine the effects of helium-neon laser and green laser on normal blood samples with comparisons using CBC and FTIR.

2. MATERIALS and METHODS

1. Individual blood samples

Blood samples from 20 healthy female volunteers were taken. 8ml of blood was extracted from each volunteer in accordance with medical laboratory standards. The samples were kept in an anticoagulant tube containing Ethylene Diamine Tetraacetic Acid (EDTA). The samples were each divided into seven smaller samples;one as a control sample while the rest were exposed either to the Helium-Neon laser or green laser separately for varying lengths of time (10, 20 and 30 minutes).

2. Laser apportionment:

As an irradiation source, the study used two types of lasers for the blood samples separately.First, a He-Ne laser pointer was used with a wave length of 650nm and a low power of 100 mW.Second, a diode green laser pointer with a 530nm wave length and a low power of 500mW was used.

3. Sample irradiation:

The samples were placed under He-Ne laser and green laser separately for 10, 20, and 30 minutes. The beam of laser was steadily pointed on the test tubes' midpoint and exposure was conducted under room temperature (18-25°C). Complete blood counts and FTIR spectrum for normal and irradiated samples were documented.

The equipment used to assess CBC values of irradiated and non-irradiated samples was automate hematology analyzer medicine (Medonic M-series M32 hematology analyzers)(Kujawa et al., 2004).FTIR was obtained using FTIR spectrophotometer (Bio-rad Merlin FTIR spectroscopy, Mod FTS 3000) for the control, He-Ne laser and green laser irradiated samples. Significance was set at P-values ≤ 0.05 .

3. RESULTS and DISCUSSION

Complete blood counts were estimated for irradiated and normal blood samples and the means of CBC parameters are shown in tables [1 and 2]. The results showed that there were slight changes that occurred in the CBC of both post- irradiated samples in comparison to the control sample but there were not statistically significant (p>0.05) differences. Table [1] demonstrates the mean results of samples after green laser irradiation were increased in (RBC, Hb, HCT, MCHC, Monocyte, granulocyte, MPV, and PDW) for different times from (4.956*1012/L, 13.2g/dl, 42.267%, 31.467g/dl, 4.633%, 63.833%, 8.667fl and 34.133%) to (5.061*1012/L, 13.520g/dl, 42.473%, 31.84g/dl, 5.83%, 69.63%, 9.44fl and 34.45%) respectively in comparison to the control sample. However, the mean of the rest of the CBC parameters (WBC, platelets, MCV, lymphocytes and RDW), with the exception of MCH and PCT, all decreased after irradiation with green laser from (8.8*109/L, 84.767fl. 278.67*109/L, 31.533%, 11.8%) to (8.72*109/L, 235.22*109/L, 84.110fl. 24.530%. 11.483%) in comparison to the non-irradiated samples the negative samples. as control

Deverse	Due investigation	Post-irradiation						
rarameters	Mean ± SD	10 min	20 min	30 min	Mean ±SD			
RBC * 10 ¹² /L	4.9567±0.388	5.023	5.0700	5.093	5.061±0.034			
WBC* 10 ⁹ /L	8.8000±2.511	8.300	9.067	8.800	8.72-±0.386			
PLT *10 ⁹ /L	278 67+42 028	236.66	234 67	234 33	235 22+1 258			
Hb g/dl	12 200+ 520	12 466	12 567	12 522	12 520+0.045			
Hct %	13.200±.329	13.400	13.507	13.555	13.320±0.043			
MCV fl	42.20/±1.380	42.200	42.667	42.567	42.473±0.241			
MCH ng	84.767±4.913	84.200	84.333	83.800	84.110±0.276			
MCHC -/-	26.700±1.276	26.866	26.867	26.633	26.783±0.132			
MCHC g/ai	31.467±0.404	31.933	31.833	31.767	31.840±0.085			
Lymphocyte %	31.533±4.105	25.533	23.700	24.367	24.530±0.926			
Monocyte %	4.633+2.542	5.766	5.733	6.000	5.830+0.147			
Granulocyte %	63 833+1 560	68 700	70 567	69 633	69 630+0 930			
RDW %	11 800 400	11.000	11.267	11.067	11 492 0 222			
PCT %	11.800±.400	11.233	11.367	11.867	11.483±0.332			
MPV fl	0.19667±0.063	0.196	0.2033	0.206	0.196 ± 0.005			
	8.667±3.214	9.266	9.467	9.600	9.440±0.170			
PDW %	34.133±16.346	33.833	34.367	35.167	34.450±0.669			

Table [1]: Hematological parameters before and after Green laser irradiation for different times (n=20)

The whole blood exposed to the (He-Ne) laser radiation mean results, as displayed in table [2], show that (RBC, Hb, HCT, MCH, MCHC, Monocyte, granulocyte, RDW, PCT and MPV) increased from (4.956*1012/L, 13.2g/dl, 42.267%, 26.7 pg, 31.467g/dl, 4.633%, 63.833%, 11.8 %, 0.196 % and 8.667fl) to (5.058*1012/L, 13.522 g/dl, 42.622%, 26.8 pg, 31.766g/dl, 5.322%, 69.566%, 11.944%, 0.212% and 8.877fl) respectively in comparison to the non irradiated whole blood samples as

the negative control samples. At the same time, there was a decrease in the mean of the samples irradiated with (He-Ne) laser for different lengths of time. The parameters (WBC, platelets, MCV, lymphocyte, and PDW) decreased from(8.8*109/L, 278.67*109/L, 84.767 fl, 31.533 %, 34.133 %) to (8.788*109/L, 257.110*109/L, 84.433fl, 26.3%, 31.999%) respectively, in comparison to the mean of the control sample.

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Parameters	Mean ± SD	10 min	20 min	30 min	Mean ±SD
RBC * 10¹²/L	4.956±.388	5.303	5.023	4.850	5.058±0.228
WBC* 10 ⁹ /L	8 800+2 511	8 766	8 833	8 767	8 788+0 038
PLT *10 ⁹ /L	278 67+42 028	223.000	232.00	316 33	257 110+51 483
Hb g/dl	12 200 + 520	14 122	12 267	12 067	12 522+ 540
Hct %	13.200±.329	14.155	15.507	13.007	15.522±.549
MCVfl	42.267±1.386	44.733	41.867	41.267	42.622±1.852
MOU	84.767±4.913	84.300	83.633	85.367	84.433±0.874
мсн рд	26.700±1.276	26.666	26.667	27.067	26.800±0.231
MCHC g/dl	31.467±.404	31.666	31.933	31.700	31.766±0.145
Lymphocyte %	31 533+4 105	22 933	22 800	33 167	26 300+5 947
Monocyte %	4 600 0 540	5 4 6 6	5.067	5 222	5 222 0 125
Granulocyte %	4.633±2.542	5.466	5.267	5.233	5.322±0.125
	63.833±1.569	71.600	71.933	65.167	69.566±3.813
RDW %	$11.800 \pm .400$	11.700	12.167	11.967	11.944±0.234
PCT %	0.196+.063	0.193	0.2100	0.233	0.212+0.020
MPV fl	8.667±3.214	9.100	9.800	7.733	8.877±1.051
PDW %	34.133±16.346	34.766	35.400	25.833±	31.999±5.349

Table [2]: Hematological parameters before and after irradiation to He-Ne laser for different time (n=20)

An increase was observed in RBC, Hb, HCT, MCHC, Monocyte, Granulocyte, and Platelets

indices due to the biostimulatory effects of the lasers on red cells with changes in the cell membrane. This, in turn, led to the growing functionality of the red cells. These findings agree with the conclusions of (Zahra Al Timimi and Jafri, 2011) that low-level laser treatment can affect the properties of blood cells, both physically and chemically.

At the same time, the means of blood parameters of each -irradiated sample (for three different durations: 10, 20, and 30 minutes) and the significance among them were obtained. As shown in table [3], slight changes were observed. Furthermore, low-energy laser irradiation has been reported to increase chemical activity and enhance the stability of RBC membranes. About 85% of lasers have thermal consequences at the tissue level. They cut, clot, evaporate, and ablate tissue from the contact site where the thermal effect comes(Kujawa et al., 2004).When the blood sample is exposed to the laser beam, the enzymatic movement of the membrane sodium (Na+) and potassium (K+) ion pump changes in quantity and fluency. As a result, the biological role of the cell is stimulated leading to an increase in light absorption. However, an additional surge in exposure constrains the enzymatic actionsas a result of the suppression of the Na+ and K+ (Haimid et al., 2019)

Parameters	Pre-irradiation	Crean lagar Maan + SD	Post irradiation	D volue
	Mean ± SD	Green laser Mean ±SD	He-Ne laserwieali ±SD	F-value
RBC * 10 ¹² /L	4.956±.388	5.061±0.034	5.058±0.228	0.989
WBC* 109/L	8.800±2.511	8.72-±0.386	8.788±0.038	0.771
PLT *109/L	278.67±42.028	235.22±1.258	257.110±51.483	0.544
Hb g/dl	13.200±.529	13.520±0.045	13.522±.549	0.995
Hct %	42.267±1.386	42.473±0.241	42.622±1.852	0.913
MCV fl	84 767+4 913	84 110+0 276	84 433+0 874	0.674
MCH pg	26 700+1 276	26 783+0 132	26 800+0 231	0.944
MCHC g/dl	20.700±1.270	20.705±0.152	20.000±0.231	0.560
Lymphocyte %	21 522 4 105	31.840±0.085	26 200 ± 5 047	0.500
Monocytes %	51.555±4.105	24.330±0.920	20.300±3.947	0.008
Granulocyte %	4.633±2.542	5.830±0.147	5.322±0.125	0.067
RDW %	63.833±1.569	69.630±0.930	69.566±3.813	0.980
PCT %	$11.800 \pm .400$	11.483±0.332	11.944±0.234	0.150
MPV fl	196±.063	0.196 ± 0.005	0.212±0.020	0.233
DDW %	8.667±3.214	9.440±0.170	8.877±1.051	0.489
	34.133±16.346	34.450±0.669	31.999±5.349	0.550

Table [3]: Comparison between average hematological results Post green laser and He-Ne laser exposure (n=20)

Table [4] and [5] shows the FTIR spectra of human blood revealing a sequence of bands with various intensities and the spectral statistics. For the methodical screening, IR spectrum is divided into three zones. Zone I ranges from 4000 to 3000 cm-1, which is the water and hydroxyl group. This zone is remarkable as it exposes the type of hydrogen bonding. Zone II ranges from 3000 to 1500 cm-1, where effective groups are monitored. In this area, significant IR absorption relating to fibrinogen takes place. Zone III ranges from 1500 - 400 cm-1, which is of considerable importance to biological minerals and their integrations(Haimid et al., 2019)

Table 4: FTIR Spectrum Analysis of blood samples with and without irradiation to green laser

Pre- and post irradiation control (1)			Pre- and post irradiation control (2)				Pre- and post irradiation control (3)					
Pre irradiation wave length cm ⁻¹ Control (1)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Pre irradiation wave length cm ⁻¹ Control (2)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Pre irradiation wave length cm ⁻¹ Control (3)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Functional group
3394	3435	3446	3412	3271	3381	3323	3414	3442	3458	3271	3334	OH
1643	1635	1653	1647	1651	1651	1643	1654	1651	1643	1643	1645	C=O
1633	1541	1637	1546	1546	1633	1633	1535	1543	1546	1614	1548	N=O
1537	1420	1544	1454	1398	1537	1548	1438	1454	1448	1543	1458	C-H
1396	1392	1398	1392	1301	1454	1396	1396	1396	1406	1444	1400	N-H
1168	1168	1230	1100	1170	1396	1303	_	-	1238	1402	1244	CO

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Pre irradiation wave length cm ⁻¹ Control (1)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Pre irradiation wave length cm ⁻¹ Control (2)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Pre irradiation wave length cm ⁻¹ Control (3)	Post irradiation wave length cm ⁻¹ (10 min)	Post irradiation wave length cm ⁻¹ (20 min)	Post irradiation wave length cm ⁻¹ (30min)	Functional group
3394	3367	3466	3412	3271	3419	3305	3332	3442	3271	3294	3332	OH
1643	1637	1647	1647	1651	1629	1643	1643	1651	1651	1643	1614	C=O
1633	1544	1546	1546	1546	1544	1546	1544	1543	1546	1544	1537	N=O
1537	1400	1398	1454	1398	1400	1458	1400	1454	1454	1400	1444	C-H
1396	1300	_	1392	1301	-	1400	1301	1396	1400	-	1396	N-H
1168	-	-	1100	1170	1170	1301	1238	-	1242	1246	1303	СО

Table 5: FTIR Spectrum Analysis of blood sample with and without irradiation to He-Ne laser.

Figure 1 shows an FTIR spectrum of whole blood that has not been exposed to radiation. Table [4] displays the groups OH, C=O, N=O, CO, and CH in the range of wave numbers 4000 cm-1 and 400 cm-1. The bonding peak of O- H is shown by wave number 3394 cm-1 of control 1. Amide-I is primarily concerned with C-O, C=O, and C-H extending vibrations, as well as the backbone's conformation. The peak points of C=O, C-O, and C-H are displayed by the wave numbers 16343 cm-1, 1168 cm-1, and 1537 cm-1. The absorption peak in the 1400cm-1 to 1200cm-1 zone is caused by the C-H deformation of the methyl and methylene proteins. C-H,

Figures (2-13) demonstrate the FTIR spectrum of normal blood exposed to green and He-Ne radiation for 10, 20, and 30 minutes . Whole blood samples are subjected to He-Ne irradiation for 10, 20 and 30 min. The FTIR spectra show that groups O-H and C=O, N=O, CH, and N-O and C-O are not present. It demonstrates that the strongest groups in blood's IR spectra are the consequence of polypeptide absorption combined with significant amounts of amino acid residues. The peptide groups' IR bands, which are assigned to stretching and bending vibrational bands of the NH, CN, and C=O bonds, enclose information regarding the conformational assembly of the spherical protein molecules. When blood

O-H, and N-H broadening vibrations of proteins are included in the 3645–3196 cm-1 zone. The N-H extending mode (amide - A) of proteins causes the high absorption peak 3466 cm-1.At approximately 2964 -2875 cm-1, the even and uneven stretching C-H vibrations of methyl and methylene are evident. At 1674 cm-1, the absorption group agrees with C=O widening vibrations (amide - I). The vibrations at 1546 cm-1, on the other hand, are associated to amide - II, which results from N-H winding vibrations that are intensely coupled with the C-N broadening of proteins(Haimid et al., 2019).

is exposed to low doses of light, the spectral fluctuations in the area of the IR spectra where the absorption bands of peptide groups appear to be more vulnerable to breaking due to the fragile intermolecular bonds appear to be more delicate to breaking. The FTIR spectra of whole blood after 30 minutes of exposure to a He-Ne laser show the groups O-H, C=O, C-O, C-H, and N=O. Because of the proteins' conformational changes, it disrupts polypeptide linkages. When blood is subjected to a green laser, groups OH, C=O, CH, and N=O are present with the exception of CO for control 3 and are absent after 30 minutes of irradiation(Ghadage and Zaware, 2018)





Figure 3: FTIR spectrum for irradiated blood (control3) with green light laser (10 min)



Figure 5: FTIR spectrum for irradiated blood (control2) with green light laser (20 min)



Figure 7: FTIR spectrum for irradiated blood (control2) with green light laser (30 min)



Figure 2: FTIR spectrum for irradiated blood (control1) with green light laser (10 min)



Figure 4: FTIR spectrum for irradiated blood (control1) with green light laser (20 min)



Figure 6: FTIR spectrum for irradiated blood (control1) with green light laser (30 min)



Figure 8: FTIR spectrum for irradiated blood (control3) with green light laser (30 min)



Figure 9: FTIR spectrum for irradiated blood (control2) with He-Ne laser (10 min)



Figure 11: FTIR spectrum for irradiated blood (control1) with He-Ne laser (20 min)

CONCLUSION

In this study, it was determined that both types of lasers have effects on the CBC tests; although not to a significant degree. During exposure to green laser light MCH and PCT were not affected. However, under He-Ne laser irradiation all CBC parameters were affected with exposure.

The FTIR spectra of normal blood laser irradiation indicated that groups O-H, C=O,C-O, NH, N=O and C-H are present. As the period of exposure to He-Ne laser irradiation on whole blood is reduced for 10, 20, and 30 minutes, the absorption for all groups, except NH and CO missing peaks due to protein denaturation for 10 and 20 minutes, respectively. However, when blood is exposed to green laser, all functional groups OH,C=O, CH and N=O are present and only peaks of CO for control 2 after irradiation for 30 min. are absent. Therefore, the findings of our research prove that green laser light therapy has a lesser effect on the blood component than He-Ne lasers.

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Figure 10: FTIR spectrum for irradiated blood (contrl3) with He-Ne laser (20 min)



Figure 12: FTIR spectrum for irradiated blood (control2) with He-Ne laser (30 min)



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